Neutrophil to lymphocyte ratio & cervical length for prediction of spontaneous preterm delivery in threatened preterm labour.

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الخلاصة:

خلفية الدراسة و الاهداف : أن القدرة على التنبؤ بالولادة المبكرة هو امر في غاية الاهمية ، لأنها قد تسمح بتحديد النساء المعرضات لخطر الولادة المبكرة و اخضاعهن للدراسات التداخلية في المستقبل، وبما ان الالتهاب يمثل احد أهم اسباب الولادة المبكرة ، فان الهدف من هذه الدراسة هو تحديد دور الواسمات البيولوجية المصلية في التنبؤ بالولادة المبكرة والجمع بين هذه الواسمات مع طول عنق الرحم لاستخدامهما كوصمة مشتركة لزيادة دقة التشخيص.

الطريقة: اشتملت هذه الدراسة المرتقبة على 80 امرأة حامل ادخلن المستشفى لكونهن مهددات بالولادة المبكرة(المجموعة الأولى) وتمت مقارنتهن مع40 امرأة في الشهر التاسع من الحمل (المجموعة الثانية) قسمن إلى مجموعتين: عشرون منهن لسن في حالة ولادة و20 أمرأة أخرى كن في حالة ولادة. تم قياس عدد الكريات البيض التبايني ومستوى البروتين المتفاعل في الدم لكل المشتركات في الدراسة وبالنسبة للمجموعة الاولى فقد تم قياس طول عنق الرحم عبر السونار المهبلي واستخراج وصمة مشتركة (نسبة الكريات البيض التبايني مقسومة على طول عنق الرحم). في نهاية الدراسة انقسمت المجموعة الاولى الى مجموعتين حسب وقت الولادة: اللواتي انجبن قبل37 أسبوع (وتشمل 30 امرأة) واللواتي انجبن بعد 37 اسبوع (و تشمل 50 امرأة).

النتائج: اثبتت الدراسة ان مستوى كريات الدم البيض التبايني ومستوى البروتين المتفاعل والوصمة المشتركة اعلى بفارق معتد به احصائيا في المجموعة الاولى عند اللواتي انجبن قبل 37 أسبوع بالمقارنة مع اللواتي انجبن بعد 37 أسبوع كما ان ان مستوى كريات الدم البيض التبايني ومستوى البروتين المتفاعل في المجموعة الثانية أعلى بفارق معتد به احصائيا عند النساء اللواتي كن في حالة ولادة بالمقارنة مع االلواتي لم يكن في حالة ولادة. يستنتج من ذلك أنه يمكن استخدام الواسمات البيولوجية المصلية كمعلمة بسيطة وحساسة لتحديد النساء المعرضات لخطر الولادة المبكرة. كما ان استخدام السونار المهبلي معها لاستخدامهما كوصمة مشتركة يزيد من دقة و حساسية كل وصمة على حدة.

Abstract:

Background: Being able to predict preterm birth is important, as it may allow a high-risk population to be selected for future interventional studies. As inflammation represents a crucial pathogenic process of preterm delivery, the aim of this study is to define the role of serum inflammatory markers in the prediction of spontaneous preterm delivery & to combine these markers with cervical length to increase the diagnostic accuracy.

Methods: Eighty pregnant women with threatened preterm labour (group I) were compared with 40 women at term (group II (, subdivided into 2 subgroups (20 of them were not in labour & the other 20 were in labour). Neutrophils to lymphocytes ratio (NLR) & C-reactive protein (CRP) level were measured & compared for all study subjects. For group I, transvaginal cervical length was measured & combined marker (defined as NLR divided by the cervical length) was estimated & they were subdivided into: (group Ia) who delivered before 37 weeks (n=30) & (group Ib) who delivered at term (n=50.(

Results: The levels of NLR (P<0.001), CRP (P<0.016) in group Ia were significantly different from those in group Ib & they were also significantly higher in group IIa than in group IIb (P<0.001). The cervix length (P< 0.001) & combined marker (P<0.001) in group Ia were significantly different from those of group Ib. It has been shown that the combined marker has higher sensitivity (65%) & specificity (87%) for

prediction of preterm delivery, as compared to cervical length or systemic inflammatory markers each alone .
Conclusions Combined marker is useful for identifying women at risk of preterm delivery in patients with threatened preterm labour .
Keywords: Neutrophil to lymphocyte ratio, cervical length, preterm delivery, threatened preterm labour.

Introduction:

Preterm birth, defined as delivery before 37 completed weeks, with an annual incidence of 9-15%, is implicated in approximately two thirds of perinatal mortality worldwide. Preterm births are also associated with neonatal death & with immediate & long-term morbidities, making this a major public health issue. For these reasons, many researchers are working to understand mechanisms of preterm birth & testing interventions to reduce its occurrence. [1, 2]

Currently, over 60% of preterm deliveries are unexplained [3], ascribable only to 'idiopathic' preterm labour or preterm premature rupture of fetal membranes. Some experts believe that these are associated with a subclinical inflammatory response in the maternal &/or fetal tissues. [4]

Furthermore, there is emerging evidence that physiological parturition is associated with up-regulation of inflammatory pathways. Labour at term is associated with a massive influx of leukocytes especially neutrophils into the myometrium & cervix. [5] Leukocyte migration during parturition appears to be actively promoted by factors released by the myometrium & cervix during labour & culminates in tissue leukocytes which are major producers of the inflammatory cytokines [6]. A role for the pro-inflammatory cytokines is evident in term & preterm delivery, & this is independent of the presence of infection. [7]

In contrast, maternal inflammatory responses (including the activation status of leukocytes) are inhibited during pregnancy. This may allow the fetal allograft to survive in a potentially immunologically hostile environment. The timing of the 'switch' whereby leukocytes of pregnant women are restored to their non-pregnant activation status is unknown. It has been hypothesized that priming & activation of maternal leukocytes in peripheral blood is a key component of parturition, & that inappropriate preterm priming of leukocytes might initiate preterm labour & delivery.[8]

Neutrophilia & lymphocytopenia – a significant increase in circulating neutrophils & decrease in circulating lymphocytes – are physiological responses of innate immune system to various stressful insults, such as systemic inflammation, malignancy, major trauma & malnutrition. [9-11]

The mechanism responsible for lymphocytopenia is induced by several factors including hormones, chemokines & cytokines, which regulate the number & activity of the lymphocyte & show the intensity of inflammation as well as the resistance & adaptability of the immune system. [10] In contrast, neutrophilia is caused by delayed apoptosis of neutrophils & stimulation of stem cells by growing factors (G-CSF). [12]

Recently, the diagnostic role of the neutrophil to lymphocyte ratio (NLR) & C-reactive protein (CRP) have been

investigated in various diseases [13-15] including preterm labour. [16-18]

In a clinical setting, accurate diagnosis of preterm labour is essential, to allow timely transfer to perinatal centers & to make informed decisions about the use of antenatal steroids & tocolytics, which can be associated with serious adverse events, in order to limit their use to cases in which they are clearly needed. [2]

Cervical length, as measured by transvaginal ultrasonography, has been shown to predict preterm birth in asymptomatic low-risk women as well as those presenting with threatened preterm labor. [19, 20]

Although the current recommendation is to combine transvaginal ultrasound measurement of cervix length & cervicovaginal fetal fibronectin for the prediction of preterm delivery, [20] more simple & sensitive methods are always needed.

Our aim is to compare the level of NLR & CRP between preterm & term patients & to show whether these inflammatory markers either alone or in combination with cervical length are useful for prediction of spontaneous preterm delivery in patients with threatened preterm labour.

Patients & methods:

We performed this prospective case controlled study in AL-Zahra'a Teaching Hospital for Maternity & Pediatrics in Najaf, Iraq, in the period from March 2012 to September 2012. The study protocol was approved by the Scientific & Ethical Committees at Kufa Medical College & an informed consent has been taken from all the study subjects.

After exclusion of major uterine or fetal anomalies & maternal & placental diseases that may alter the immune response including any infective &/or inflammatory process, DM, pregnancy induced hypertension, intrauterine growth restriction, placenta previa or abruption, a total of 120 women with singleton pregnancy were enrolled in the study & divided initially into 2 groups.

(Group I): including patients with threatened preterm labour (n=80).

All patients in this group fulfilled the following criteria:

1. Gestational age between 24 -36 weeks.
2. Presence of regular uterine contractions (8 or more in 60 minutes).
3. Initial cervical dilatation < 3 cm by digital cervical examination with intact membranes.
4. The absence fever & fetal tachycardia.
5. No history of prior or present cervical cerclage.

Group II: including healthy women at term (37-42 weeks), subdivided into 2 subgroups: (group II a) were not in labour (n=20) & (group II b) were in early labour with intact memberanes (n=20).

For all study subjects, Gestational age was based on the date of last menstrual period & confirmed by first trimester ultrasound scan.

Neutrophil & lymphocyte counts & CRP levels were measured in group I at admission & compared with those obtained for group II.

Leucocyte differential counts were analyzed by (SIEMENS ADVIA 2120 system). The neutrophil to lymphocyte ratio (NLR) was defined as the absolute neutrophil count divided by the absolute lymphocyte count.

CRP level was measured by ELISA by High-Sensitivity CRP ELISA Kit (CircuLex).®

Patients in group I, were admitted to the hospital for observation & follow up, & if contractions did not improve with hydration, tocolysis (nifidipine) along with i.m corticosteroids were given. Cervical length measurement was performed to all patients in this group by the same skilled sonographer. Transvaginal ultrasonographic assessment of cervix length was performed with a 6-MHz transducer. After micturition, in lithotomy position transvaginal probe is placed in the anterior vaginal fornix, & cervix length was defined as the distance between the internal & external cervical os. The shortest distance of three measurements was used as cervix length. If the examiner observed a uterine contraction during the examination, the examination stopped until the contraction resolved.

The combined marker was calculated for patients in group I which is defined as NLR divided by the cervical length. At the end of the study, group I was subdivided into 2 subgroups according to the time of delivery: those who delivered before 37 completed weeks (n=30) & those who delivered ≥ 37 weeks (n=50). NLR, cervical length & the combined marker in (group I a) were compared with those in (group I b).

Statistical analysis: All analyses were performed using (SPSS-*17*) in which we measure mean & standard deviation for different measurement data. Independent sample t-test & ANOVA test were used to measure the difference between the groups. Receiver operating characteristic (ROC) curve was used to measure the optimal cutoff level. We set p-value <0.05 as significant.

Results:

Clinical characteristics of the study population are shown in Table (1). No significant difference in the age, body mass index (BMI), gravidity & parity among the 4 study groups. However the gestational age on admission was significantly different among the studied groups (p <0.001).

Table (1): Clinical characteristics of study subjects.

	Group Ia (n=30)	Group Ib (n=50)	Group IIa (n=20)	Group IIb (n=20)	P-value
Age (yr)	28.03±4.58	26.02±3.94	25.70±3.04	24.70±5.07	0.028
BMI (kg/m^2)	23.76±1.40	24.19±2.25	22.05±1.54	23.8±2.39	0.88
Gravidity	2.50±1.28	2.58±1.21	2.92±1.56	3.14±0.25	0.68
Parity	1.28±0.23	1.15±0.25	1.17±0.27	1.04±0.23	0.76
GA (week)	31.03±2.24	30.96±2.53	38.1±0.97	37.5±0.68	0.001

Group Ia=patients with threatened preterm labour who delivered < 37 week, Group Ib=patients with threatened preterm labour who delivered at term, Group IIa= women at term in labour, Group IIb: women at term not in labour, GA= gestational age at sampling.

Table (2): Comparison of neutrophil count, lymphocyte count, CRP & NLR among the study groups.

	Group Ia (n=30)	Group Ib (n=50)	Group IIa (n=20)	Group IIb (n=20)	P-value
Neutrophil (X 10^9/L)	9.79±0.73	7.22±0.81	8.42±1.27	6.5±0.70	0.001
Lymphocyte (X 10^9/L)	1.42±0.32	1.66±0.42	1.89±0.24	1.77±0.27	0.001
NLR	7.29±2.07	4.57±1.13	4.5±0.70	3.74±0.70	0.001
CRP (mg/dl)	1.48±0.30	0.82±0.26	1.17±0.27	0.81±0.21	0.001

Group Ia=patients with threatened preterm labour who delivered < 37 week, Group Ib=patients with threatened preterm labour who delivered at term, Group IIa= women at term in labour, Group IIb: women at term not in labour, NLR=neutrophil to lymphocyte ratio, CRP= C-reactive protein.

Analysis of data in (table 2) revealed the following:

- ***Neutrophil count*** in groups Ia, Ib, IIa & IIb were (9.7, 7.2, 8.4 & 6.5) respectively. The count was significantly higher in group Ia than group Ib (P < 0.001), IIa (P < 0.001), & IIb. It was also significantly higher in group IIa than IIb (P<0.01).
- ***Lymphocyte count*** in groups Ia, Ib, IIa & IIb were (1.4, 1.6, 1.8 & 1.7) respectively. The count was significantly lower in group Ia than group Ib (P < 0.01), IIa (P < 0.001), & IIb (P < 0.01). It was also significantly lower in group IIa than IIb (P < 0.01).
- ***The NLR*** in groups Ia Ib, IIa & IIb were (7.2, 4.5, 4.5 & 3.7) respectively. It was significantly higher in Group Ia (P < 0.001) than group Ib (P < 0.001), IIa (P < 0.001) & IIb (P < 0.001). It was also significantly higher in group IIa than IIb(P < 0.001) .
- ***Serum CRP*** in groups Ia, Ib, IIa & IIb were (1.48, 0.82, 1.20 & 0.81) respectively. Its level in group Ia was significantly higher Ia than group Ib (P < 0.001), IIa (P < 0.001) & IIb (P < 0.01). It was also significantly higher in group IIa than IIb. (P < 0.001).

For patients in group I (threatened preterm labour), comparison of CRP, NLR, cervical length & the combined marker between Group Ia (those who delivered before 37 weeks & Group Ib (those who delivered ≥ 37 weeks) were done. It has been shown that the level of CRP & NLR is significantly higher in Group Ia than in group Ib (p<0.0001), the cervical length is significantly shorter in Group Ia than in group Ib (p<0.0001) & the result of combined marker is significantly lower in Group Ia than in group Ib (p<0.0001) as shown in (table 3) .

Table (3): Comparison of CRP, NLR, cervical length & the combined marker between Group Ia & Group Ib.

	Group Ia (n=30)	Group Ib (n=50)	P-value
CRP (mg/dl)	1.48±0.30	0.82±0.26	0.0001
NLR	7.29±2.07	4.57±1.13	0.0001
Cervical length (cm)	2.21±0.44	3.51±0.34	0.0001
Combined marker	0.319±0.098	0.82±0.24	0.0001

Group Ia=patients with threatened preterm labour who delivered < 37 week, Group Ib=patients with threatened preterm labour who delivered at term, NLR=neutrophil to lymphocyte ratio, CRP= C-reactive protein.

The diagnostic value (sensitivity, specificity, positive & negative predictive values) of neutrophil & lymphocyte counts, CRP, NLR, cervix length & combined marker in predicting spontaneous preterm delivery <37 weeks in patients with threatened preterm labour (group I) was measured & compared using ROC curve analysis (Table 4). The following results were noted:

- **Combined marker** had the highest AUC. The AUC for combinead marker was 0.83 (95% CI: 0.74–0.89) with sensitivity of 65.6% & specificity of 87.8% at a cut-off value of 0.295.
- For **NLR**, the sensitivity was 51.9% & the specificity was 73.1% at a cut-off value of 5.47.
- For **cervical length** the sensitivity was 60.1% & specificity was 79.8% at a cut-off value of 1.7 cm.
- For **neutrophil count,** the sensitivity was 41.1% & specificity was 88% at a cut-off value 9.55.
- For **lymphocyte count,** the sensitivity was 39.9% & specificity was 86.4% at a cut-off value 1.35.

Table (4): Comparison of diagnostic usefulness of neutrophil & lymphocyte counts, CRP, NLR, cervix length & combined marker in patients with threatened preterm labour.

	AUC(95%CI)	Sensitivity	Specificity	PPV	NPV	Cut-off value
Neutrophil	0.59 (0.52-0.63)	0.411	0.88	0.834	0.512	9.55
Lymphocyte	0.59 (0.51-0.68)	0.399	0.864	0.731	0.52	1.35
Cervical length	0.75 (0.68-0.82)	0.601	0.798	0.714	0.634	1.7
CRP	0.69 (0.58-0.79)	0.612	0.677	0.742	0.571	0.44
NLR	0.64 (0.54-0.77)	0.519	0.731	0.775	0.548	5.47
Combined marker	0.83 (0.74-0.89)	0.656	0.878	0.856	0.645	0.295

*AUC= area under the curve; CI= confidence interval; CRP= C-reactive protein; NLR=neutrophil to lymphocyte ratio; PPV=positive predictive value NPV=negative predictive value.

Discussion

Several strategies for the identification of women at risk of preterm delivery have been proposed, such as risk scoring systems, biochemical markers of inflammation, cervical length & fetal fibronectin. These were developed to decrease unnecessary interventions for patients with symptoms of preterm labour & to identify patients who might benefit from aggressive therapy that would include tocolysis, corticosteroids, & transfer to a tertiary care facility. [21]

Risk scoring systems are based on either risk factors (e.g. socioeconomic history, lifestyle) or clinical information (e.g. obstetric history). The predictive value & specificity of these scores are poor mainly because many of these risk factors are indirect. [22]

Transvaginal sonographic measurement of cervical length has been shown to be an objective, reproducible & reliable method to predict preterm delivery in high risk pregnancies, however it has low sensitivity & specificity. [20, 23, 24, 25]

The selective use of fetal fibronectin detection after cervical length measurement has been found to be more specific than cervical length alone in predicting the risk of spontaneous preterm delivery in threatened preterm

labour Although, negative fetal fibronectin are able to stratify females who will not deliver prematurely after the onset of symptoms, a positive test is only a moderate predictor of spontaneous labour before 37 weeks of gestation. [20, 26, 27]

Hence there is an urgent need to discover reliable indicators of preterm labour. Inflammation has been implicated in the mechanisms responsible for preterm & term parturition, as well as fetal injury.[28] Although inflammatory markers as combination of cervical interleukin-6 appears to be promising in predicting preterm birth, but the relatively low sensitivity restricts its clinical utility. [28, 29] Furthermore we need a test examined easily in clinical practice, & the result of the examinations must be obtained quickly in order to assess the risk of preterm delivery & to decide whether or not some treatment should be initiated. So far maternal blood test (as white blood cells count, serum CRP) has been routinely used in clinical practice to identify risks for spontaneous preterm delivery in patients with threatened preterm labour. [18, 30-35]

Our study revealed that patients with threatened preterm labour who delivered before 37 weeks have relative neutrophilia , lymphocytopenia & increased NLR as compared to those who delivered ≥ 37 weeks. These findings were consistent with Yuan et al, [8] Karen et al,[31] Romero et al[34], & Min-A Kim et al.[35] Our data also showed that neutrophil counts increased & lymphocyte counts decreased; with significantly increased NLR in patients with labour at term confirming that inflammatory mediators play a crucial role in human parturition. These findings were in agreement with Siegel et al, [36] Yuan et al [8] &Thomson et al [37].

CRP is an acute-phase protein mainly synthesized by the liver in response to some cytokines. They are potentially useful to diagnosing infection & monitoring different clinical conditions & it is also altered during pregnancy, labor, & postpartum in normal conditions & disease. In our research the mean CPR is found to be significantly higher at term in laboring women than those who were not in labour, in consistence with Luciane et al. [38] We also found that the CRP was significantly higher in patients who had spontaneous preterm delivery < 37 weeks than those who delivered ≥ 37 weeks, this goes with Cammu et al [17] Waranuch,[30] Hvilsom et al,[33] & Min-A Kim et al[35]

However the diagnostic value of neutrophil & lymphocyte counts, NLR & CRP in the prediction of preterm delivery in patient with threatened preterm labour were not promising with sensitivity & specificity were for neutrophil count 40% & 88%, for lymphocyte count 39% & 86% , for CRP 61% & 67% & for NLR 51%,73%) respectively.

In women with threatened preterm labour, sonographic measurement of cervical length helps distinguish between true & false labor & there is an inverse correlation between cervical length & the frequency of preterm delivery.[35] Our data revealed that transvaginal sonographic cervix length measurement provides a more valuable method for predicting spontaneous preterm delivery < 37 weeks in patients with threatened preterm labour with sensitivity & specificity of 60% & 79% respectively, these figures were within the ranges found by the previous studies of Tsoi et al,[24] Sotiriadis et al,[25] Honest et al[26] & Min-A Kim et al.[35]

As no single biomarker has yet been shown to fulfill all the requirements of a clinically reliable predictive test for spontaneous preterm birth ,multiple-marker tests based on combinations of several independent markers or adding their results to cervical length test results may enhance our ability to predict spontaneous preterm birth. The combined marker obtained by dividing cervix length by NLR, has been suggested as a simple novel marker for spontaneous

preterm delivery in patients with threatened preterm labour.

In our study the combined marker was significantly higher in patients who delivered < 37 weeks than those who delivered ≥ 37 weeks of gestation. When compared to cervix length or NLR alone, the combined marker has the highest AUC 0.832 (95% CI: 0.748–0.899) with sensitivity of 65% & specificity of 87% at a cut-off value of 0.29. These results indicate that the combined marker has better diagnostic value for patients with preterm delivery than cervix length or NLR alone. This finding was supported by Sorahiro etal, [23] Min-A Kim et al, [35] Conde-Agudelo et al [39] & Goldenberg et al.[40]

Conclusions: our study revealed that the combined marker is more sensitive & specific for predicting spontaneous preterm delivery in patients with threatened preterm labour in comparison with cervix length, CRP & NLR alone. The combined marker is a cost-effective test as it is based on leucocyte counts & cervical length which are already investigated in patients with threatened preterm labour.

Recommendations: larger randomized controlled trials are needed to confirm the clinical usefulness of this marker of spontaneous preterm delivery.As women with preterm contractions are often worried that these contractions represent actual preterm labor, the image of cervical ultrasound & the level of the combined marker may be used to counsel them about the risk of spontaneous preterm delivery.

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